

## Changes Produced In Cerebellum And Kidney of Rabbits After Chronic Exposure To Mobile Phone Microwaves. A Histopathological Study

Samir A. Nassar\* and Marium O. Shkorffo\*\*

\*Zoology Department, Faculty of Science, Zagazig University, Egypt

\*\*Biology Department, Faculty of Arts and Science  
(AL-Khoms), Al-Merbeh University, Libya

### Abstract

**Background:** The use of mobile phones is currently one of the fastest growing technological developments. The microwave emitted by these telephones are considered as one of the non-ionizing radiations which still having much uncertainties about the severity of effects of both acute and chronic exposure to their various types. The direct biological effects of exposure to this kind of ionizing radiation have not been studied extensively particularly from the histological point of view. The close proximity of the antenna of such a device to the head and the abdominal organs has raised concerns about the biological interactions between these Electromagnetic Radiation (EMR) and cerebellum and kidney. So these organs were chosen to be target organs for our present study.

**Aim:** The present work was performed to assess and investigate the histopathological effects of the frequent and long term exposure to the microwaves emitted by the mobile phone on cerebellum and kidney of rabbits.

**Materials & Methods:** Male and female rabbits have been used in this experiment for normal and exposed groups. Animals were sexually immature and classified into 3 groups: Normal control (non-exposed) rabbits (♂, ♀), Irradiated (exposed) ♀ rabbits, Irradiated (exposed) ♂ rabbits. The radiation exposure was carried out on heads of animals for 30 min/day for a period of 90 days at the frequency 900 MHz (Specific Absorption Rate "SAR" was 0.62 W/kg). For kidney the duration of exposure was 90 min/day for a period of 90 days at the same range of frequency but the device was operated but not activated.

**Results:** The exposure of the experimental animals of both sexes to this type of non-ionizing radiations resulted in many histopathological alterations in both cerebellum and kidney. In cerebellum herniation of some cerebellar folia, detaching of epithelia of the pial surface and generalized perineural, perivascular and perigial edema could be recorded. The Purkinje cells appeared degenerated, sometimes highly destructed, irregular in shape, dark in staining, small in size, ill-defined and surrounded by widened preicellular spaces. In some regions of cerebellar tissue an absence of Purkinje layer after the degeneration of their cells was detected. The granular cells appeared in darkly stained, clusters aggregated as gliosis, small in size with hyperchromatic pyknotic nuclei. The granular cell layer in some fields accepted a generalized spongiform appearance resulted in compression and degeneration throughout the cerebellar cortex. The molecular layer contained few destructed nerve cells, vacuolated matrix and sometimes infiltrated with degenerated dark cells. In some examined fields it accepted a spongiform appearance after severe damage to its constituent fibers and cells. The renal tissue exhibited pronounced tubular necrosis, vacuolar degeneration in glomeruli, narrowing of the Bowman's space, partial necrosis in the medullary elements with some pyknotic nuclei in the interstitium, tubular collapse, atrophied renal epithelia of the renal tubules forming necrotic remnants, glomerular sclerosis, the renal tubules may be reduced to collapsed skeletons, desquamation and absence of the normal renal epithelia. The distal convoluted tubules were more sensitive and more affected than the proximal convoluted tubules. In both of the tested organs (cerebellum, kidney) the effects of EMR were more destructive and more adverse in irradiated males than irradiated females.

**In conclusion:** the chronic exposure to the radiofrequency radiation of the mobile phone resulted in many histopathological alterations in cerebellum and kidney. The subject which

leads us to suggest that these radiations may be neurotoxic and nephrotoxic at least under the conditions used in the present experiment (30 min/day for cerebellum and 90 min/day for kidney for a period of 90 days at SAR value of 0.62 W/Kg).

**Key words:** Mobile phone microwaves- cerebellum- kidney- rabbit.

## Introduction

The growing use of digital mobile phones, using protocols such as GSM (Global System for Mobile communication) has recently stimulated discussion about the possible health effects of the radiofrequency electromagnetic field (RF Field) emitted by these phones. The microwaves emitted from the mobile phones is a type of non-ionizing radiations (NIRs) which still having much uncertainties about the severity of effects of both acute and chronic exposure to their various types. Their possible effects on the target organs such as the central nervous system and kidney have to be tested. Some studies on animals have shown that chronic exposure to mobile phones failed to induce central nervous system tumors (*La Regina et al., 2003 and Shirai et al., 2007*). *Sanchez et al. (2006)* reported that chronic local exposure to non-ionizing radiations of mobile telephone (GSM 900 or 1800) didn't demonstrate major histological variations in skin of hairless rats. On the contrary, radiofrequencies of cellular phones may affect biological systems, by increasing free radicals (enhancing lipid peroxidation) and by changing the antioxidant defence system of tissue, thus leading to oxidative stress (*Ilhan et al., 2004 and Meral et al., 2007*). Microwave exposure in the frequency and intensity of mobile telephony (900MHz band) was unlikely to produce pathologically significant changes of the blood brain barrier permeability as reported by *Fritze et al., (1997)*. They exposed rats to these microwaves for a duration of 4h. at specific brain absorption rates (SAR) of 0.3-7.5 W/Kg. and recorded this effect. *Inaloz et al. (1997a)* recorded different histopathological changes in the pregnant and new born rats placed next to the closed door of a microwave oven. They noted congested vessels, edema and degenerated neurones in all experimental dams. Progressive edema and conspicuous congested vessels were also seen in the

offspring of both experimental groups. A pathologic leakage across the blood-brain barrier which might be combined with damage to the neurones in the rats exposed for 2 hr to mobile phone electromagnetic fields of different strengths was recorded by *Salford et al. (2003)*.

The effects of the radiofrequency radiation on the kidney appears to be one of the most important issues to be studied. *Inaloz et al. (1997b)* studied the effects of microwave radiation on pregnant and newborn rat kidneys. They observed an enlarged space in the Bowman's capsule and slightly swollen glomeruli in the experimental dams. They also recorded necrosis of the renal tubules of experimental litters, and found a positive correlation between the duration of exposure and the severity of tubular necrosis. The effect of low intensity microwave radiation on the rat kidney was studied by *Nergiz et al. (2000)*. They recorded the harmful effects of these radiations on kidney parenchyma in an exposure-period dependant manner. They also examined the renal tubular epithelia and glomeruli which reflected features of early necrotic changes. *Athina et al. (2004)* recorded that at the histological level, kidney sections of exposed newborn rats occasionally exhibited a slight hydropic swelling in the tubular cells when compared to the corresponding tissue from the control animals. The exposure was of pregnant rats to pulsed GSM-like radiofrequency radiation (9.4 GHz). An increase in malondialdehyde (MDA), N-acetyl- $\beta$ -D-glucosaminidase and a decrease in glutathione levels in different tissues of rats including kidney was recorded by *Ozguner et al. (2005)* due to long term mobile phone exposure. Treatment with caffeic acid phenethyl ester (CAPE) restored the normal values. The present study aims at the evaluation of the histopathological effects of the frequent and long term exposure to mobile phone microwaves on cerebellum and kidney of

the domestic rabbits. Therefore, we attempt to hit the biological risks of these non-ionizing radiations upon the general health taking the histopathological alterations of cerebellum and kidney as bioindicators to whether confirm or expel this danger as well as to introduce a consistent and convincing scientific evidence of adverse health effects caused by these radiations.

## Material and Methods

### Animals:

Twelve male and female domestic rabbits (*Oryctolagus cuniculus*) of the same generation were obtained from the animal house at the Faculty of Pharmacy, EL-Fateh University, Libya. Animals were sexually immature (one month old). The external genitalia of males were not detected yet at the beginning of the experiment. They were allowed three days to acclimatize. Standard pellets of rabbit were provided by the animal house as a food for normal and experimental animals. Clean natural water was given ad libitum.

### Animal Grouping:

Animals were divided into three groups, four animals in each one.

*Group i:* The normal control (un-exposed group) animals of both sexes.

*Group ii:* The group of irradiated (exposed) females (four).

*Group iii:* The group of irradiated (exposed) males (four).

### Cages:

Animals were housed in living cages under normal temperature, pressure, humidity, good ventilation and day and night illumination cycle. Three large similar cages were used as ordinary living cages (dimensions 75 × 50 × 30 cm). A fourth cage was designed to lodge one animal only during the time of mobile phone microwave exposure (exposure cage) where the animal was not freely able to change its position (dimensions 28 × 12 × 10 cm).

### Mobile phone characteristics:

The apparatus is GSM (digital) class, Model Nokia 5210 made in China, 3.6 v. CE 168, Code: 0511752, Frequency: 900

MHz band, SAR rating: 0.62 w/kg, Dimensions: (106 × 48 × 22 mm), Weight: 92 g. Two mobile phones were used (both of Nokia 5210 model) in the present work, one was used as a signaling one. The other one was receiving the calls in the exposure system.

### Irradiation Technique:

During exposure each animal was placed separately in the exposure cage. The cellular phone was placed above the cage 0.5 cm distant from the right side of the brain of the irradiated animal. The mobile phones were in standby position. Animals of both sexes were exposed to the non-ionizing radiations of the mobile phone seeking for any sexual differences, at the cellular level, in response to these non-ionizing radiations. The head of each animal in the two exposed groups (both males and females) was exposed to microwaves (frequency 900MHz, SAR rating 0.62 W/Kg) emitted by the activated mobile phone for 30 min./day for 90 days taking the cerebellum as a target organ. Concerning the kidney, the mobile phone was fixed at the lateral wall of the exposure cage nearly corresponding to the right kidney of the animal in a standby position (operated but not activated by the other phone) for 90 min/ day for 90 days.

### Tissue specimens:

Normal and treated rabbits were sacrificed by cervical decapitation small pieces of right cerebella and right kidneys of the normal and irradiated animals were removed, fixed in 10% neutral formalin, washed, dehydrated, cleared and finally embedded in paraffin blocks. Thin sections (4μ) were prepared for microscopic examination. Light microscopy was performed after slides were routinely stained with haematoxylin and eosin according to the method of Bancroft and Gamble (2002).

## Results

### i- cerebellum:

In comparison with the cerebellum of normal control animals (figs.1-3) the histological examination of tissue sections obtained from the cerebellar cortex of irradiated animals (females & males)

revealed several histopathological lesions:

*a- Irradiated ♀ rabbits:*

At low magnification (Fig. 4) the field illustrated a preserved histological pattern of the three cortical layers of cerebellum, but despite this, an obvious herniation of some cerebellar folia could be demonstrated due to the increase in width and thickness of the granular layer. Also the epithelial cells of the pial surface underwent a state of detaching. In addition, degeneration and discontinuity could be noted in parts of the Purkinje cell layer with a consequent loss of these neurons. The light microscopic examination of further sections of the cerebellar tissue at higher magnifications (Figs. 5 - 7) illustrated more clearly the dangerous effects of the non-ionizing radiations emitted by the mobile phone. Fig (5) revealed part of the three cortical layers with three Purkinje cells ( $P_1$ - $P_3$ ) at different stages of degeneration.  $P_1$  having distorted outline and ill-defined nucleus,  $P_2$  underwent complete disruption and degeneration, while  $P_3$  appeared having irregular shape with complete absence of nuclear material. The cells of the granular layer appeared degenerated and accumulated in darkly-stained clusters beneath the Purkinje cell layer. The molecular layer appeared vacuolated. In another field (Fig. 6) the Purkinje cells appeared highly degenerated. Some areas seemed to be devoid of them with a resultant loss in these neurons at that area of the tissue. The cells of the granular layer appeared greatly damaged and clumped in groups enclosing edematous spaces congested with blood. The molecular layer contained few destructed and scattered nerve cells. The microscopic examination, by the oil immersion lens ( $\times 1000$ ), disclosed deleterious effects of microwave irradiation (Fig.7). The field of the cerebellar cortex contained a dark Purkinje cell exhibited an obvious state of nuclear and cytoplasmic degeneration, showed a distinct chromophilia and surrounded by aggregates of severely destructed granular cells. The observed edematous spaces resulting into compression of the neuronal mass with cellular crowding, degeneration and gliosis at some places. In the same field, increased and widened pericellular spaces could be demonstrated.

*b- Irradiated ♂ rabbits:*

The histological examination of sections in cerebellar cortex of the microwave exposed ♂ rabbits indicated a much more complicated picture where the effects of these non-ionizing radiations were more adverse and more destructive (Figs.8-11). The light microscopic examination at low magnification (Fig. 8) revealed a marked disturbance in the pattern of foliation in cerebellum as compared to those of the normal animals. Besides herniation, the three cortical layers demonstrated some sort of cavitation inside their matrix and between the molecular and the granular layers. Also the field illustrated the degeneration and absence of Purkinje cells, at several sites of their layer, as a result of irradiation. In A magnified part (Fig. 9) of the previous field showed four dark and ill-defined Purkinje cells. They were smaller in size than their corresponding cells in the normal and exposed ♀ animals at the same magnification. They appeared without any cellular details and showed different degrees of deterioration. They were separated from the underlying granular cells by increased and widened pericellular spaces. The granular cell layer contained a large number of degenerated cells with pyknotic nuclei forming aggregates or clusters. Also, the cells of the granular layer appeared smaller in size than their comparable cells of the normal and exposed ♀ animals. They appeared deeply stained and degenerated with hyperchromatic pyknotic nuclei. Also most of them underwent apoptosis. In another field (Fig.10) of cerebellar cortex of microwave exposed ♂ rabbit subjected to examination at higher magnification of Fig. (8), the field showed parts of the granular, Purkinje and molecular layers exhibited a generalized spongiform alterations resulting into compressive and degenerative changes in the neuronal mass. In the field one could demonstrate highly destructed Purkinje cells leading to an absence or complete loss of these cells in this layer. Thus there was a generalized picture of compression and degeneration throughout the cerebellar cortex due to spongiform changes, i.e. generalized perinuronal, perivascular and perigial oedema as well as oedema in the pericellular spaces. A magnified part of the

outer molecular layer in Fig. (8) showed a highly vacuolated matrix with numerous vacuoles of different sizes and few damaged cells with pyknotic nuclei. The field also illustrated an infiltration of dark cells within the fenestrated matrix of this layer (Fig.11).

## ii- Kidney:

The light microscopic examination of sections in kidney of irradiated animals of both sexes exhibited many histopathological changes:-

### a- Irradiated ♀ rabbits :

The examination of the tissue sections of kidney of female rabbits exposed to microwaves of mobile phone at different magnification powers revealed several histopathological alterations (Figs. 15-17). Fig. (15) illustrated part of the renal cortex contained two renal corpuscles with hypertrophied glomeruli having degenerated and pyknotic nuclei. The cells of distal convoluted tubules (DCT) appeared with degenerated cytoplasm and marked signs of karyolysis in some of their nuclei. Some proximal convoluted tubules (PCT) cells showed pyknotic nuclei, while others exhibited degenerated cytoplasm and nuclei. All the previous changes suggest a state of tubular necrosis. Also some sort of adhesion was noted between the glomerular tuft and Bowman's capsule. The cells of macula densa, at the vascular pole of the renal corpuscle, which characterized by the close proximity of its nuclei appeared with degenerated nuclei. In another field of kidney sections (Fig.16) all the tubular elements of the renal medulla revealed partial cellular necrosis with some cells contained pyknotic nuclei. Others showed signs of karyorrhexis and vacuolated cytoplasm. In Fig. (17) the renal tubules revealed an obvious state of tubular collapse, and moderate inflammatory infiltrate in the interstitium. Most of PCT and DCT cells appeared highly affected. But the DCT cells were more sensitive than that of the proximal ones. Some of the epithelial cells lining the DCT contained pyknotic nuclei while others are completely destroyed. Cells lining PCT showed marginal chromatin or condensed chromatin

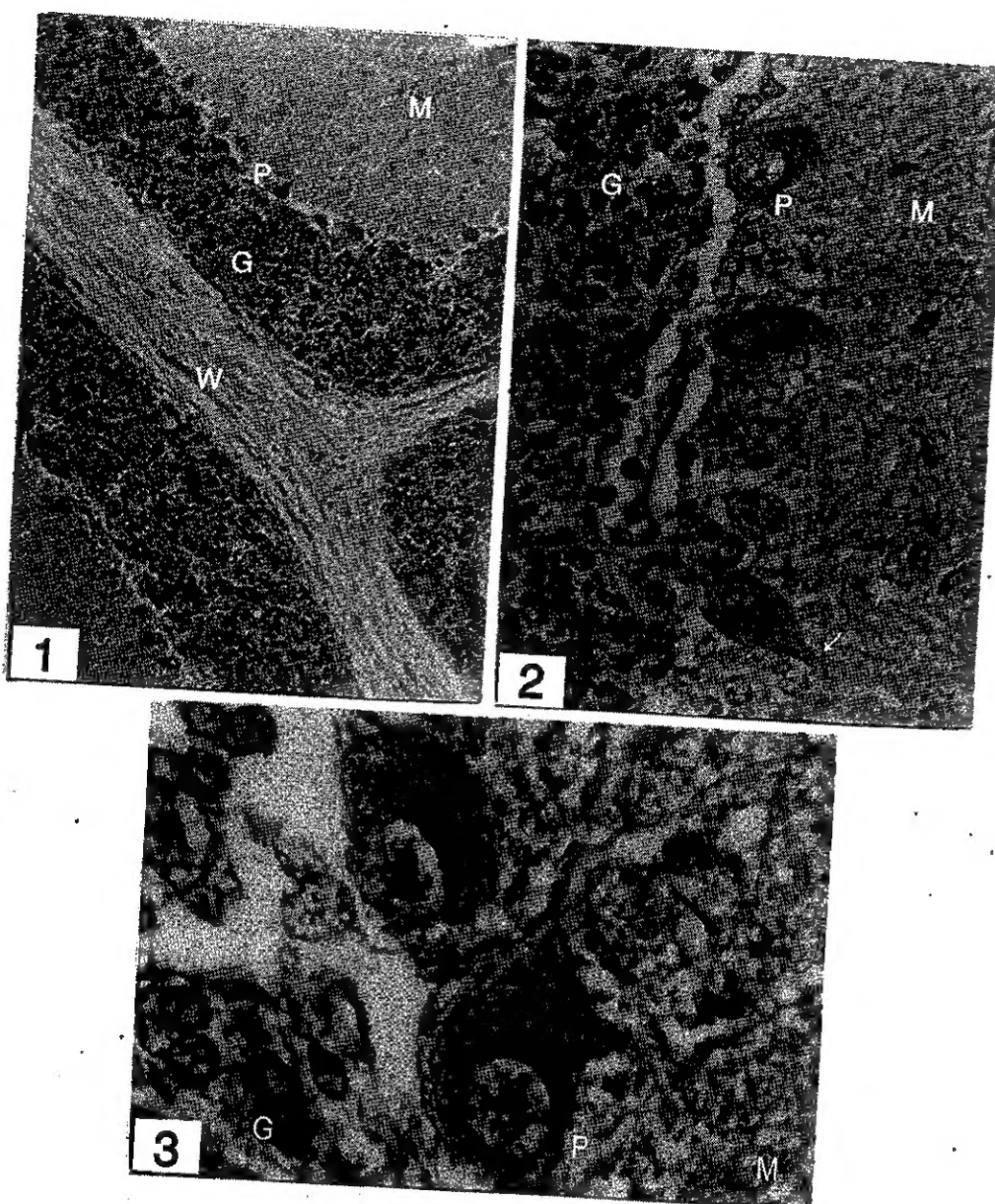
(pyknosis) while others appeared with karyolysed nuclei. So the histological structure of the tubular elements showed an obvious necrosis. The examination of another field of the kidney of irradiated ♀ rabbits at higher magnification (Fig. 18) disclosed a hypertrophied renal corpuscle with a vacuolar degeneration in its glomerulus, degenerated and pyknotic nuclei, highly affected visceral and parietal layers and complete adhesion between the glomerular tuft and Bowman's capsule leading to the absence of the renal space. The epithelia of the adjacent renal tubules appeared atrophied, vacuolated and containing necrotic remnants.

### b- Irradiated ♂ rabbits:

The examination of the tissue sections of kidney of irradiated male rabbits revealed a much more complicated destructive phase of non-ionizing radiations on the renal tissue (Figs. 19-21). In Fig.(19) the field demonstrated a part of the renal cortex exhibiting a renal corpuscle with glomerular sclerosis at the proximity of the vascular pole. The cells of the proximal and distal convoluted tubules showed a high degree of degeneration in their nuclei and cytoplasm progressed to a marked tubular necrosis and collapse. In the field another two highly degenerated renal corpuscles could be noted. In Fig. (20) the examination recorded severely damaged renal corpuscles, tubular necrosis, tubular atrophy with complete absence of the normal tubular epithelia due to necrosis and desquamation of epithelial cells. Some tubules appeared reduced to collapsed skeletons of basement membrane containing cell remnants. The tubular atrophy resulted in widened intertubular spaces and cellular debris. In another field of renal cortex (Fig. 21) the renal tissue appeared severely affected. The renal corpuscles were completely damaged. The DCT, PCT and descending limbs of Henle's loops appeared highly necrotic and atrophied. The renal parenchyma appeared evacuated and depleted of the renal elements and contained many cellular debris due to the high grade of the tubular atrophy resulted from irradiation. Here also, it could be recorded that the DCT were more affected than PCT.

Explanation of figures:

Plate-I



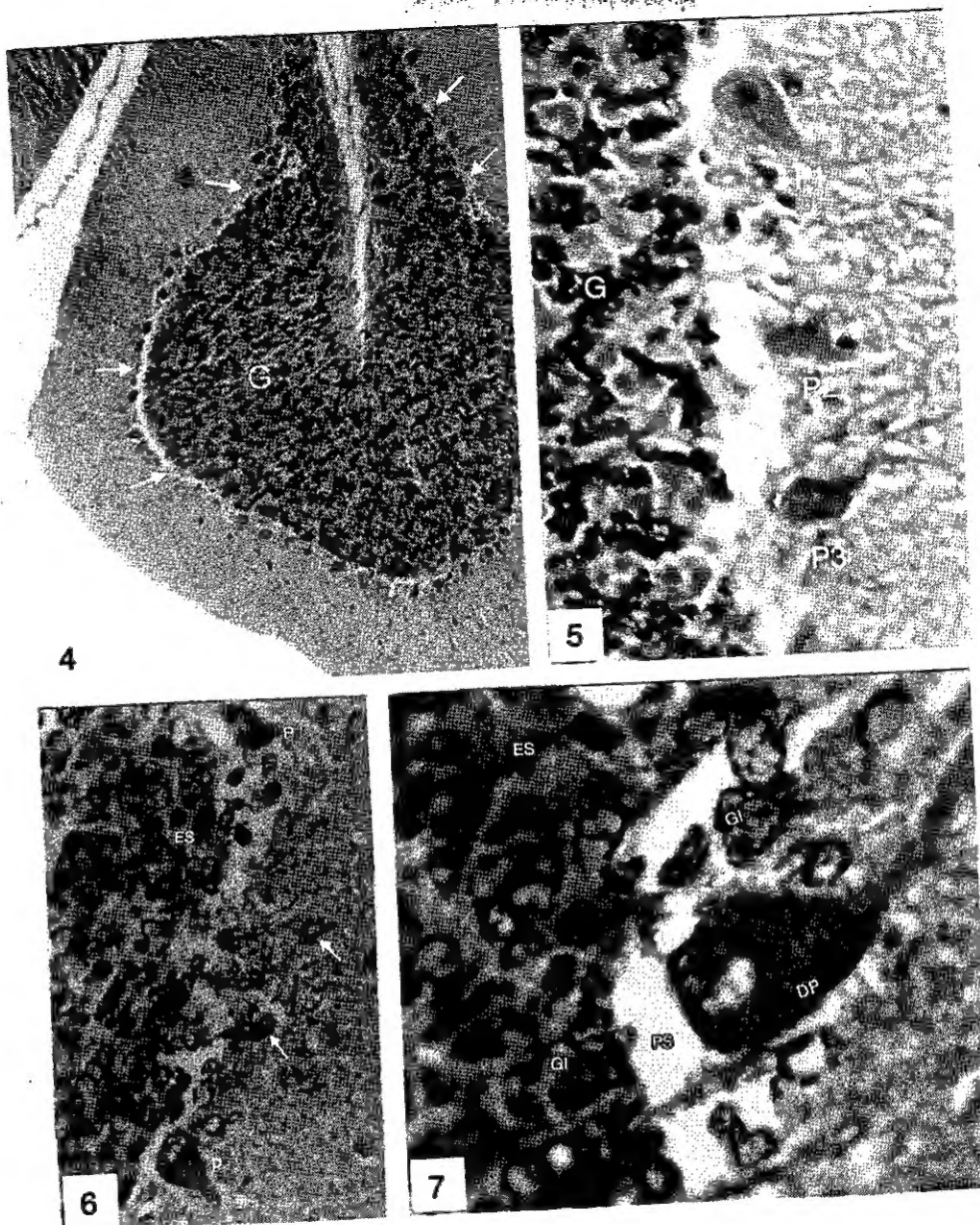
**Photomicrographs (Figs. 1-3) of sections in cerebellar cortex of normal control rabbits stained with H. & E.:**

Fig.(1): Showing the characteristic pattern of cortical layers: the molecular layer (M), Purkinje cell layer (P), granular layer (G) and core of white (w) matter ( $\times 200$ ).

Fig.(2): Showing the lightly stained molecular layer (M) with few scattered neurons, Purkinje cells (P) aligned in a monolayer between the molecular and the granular layers one of them having an apical cone (arrow) and the granular layer (G) with its darkly-stained groups of neurons ( $\times 400$ ).

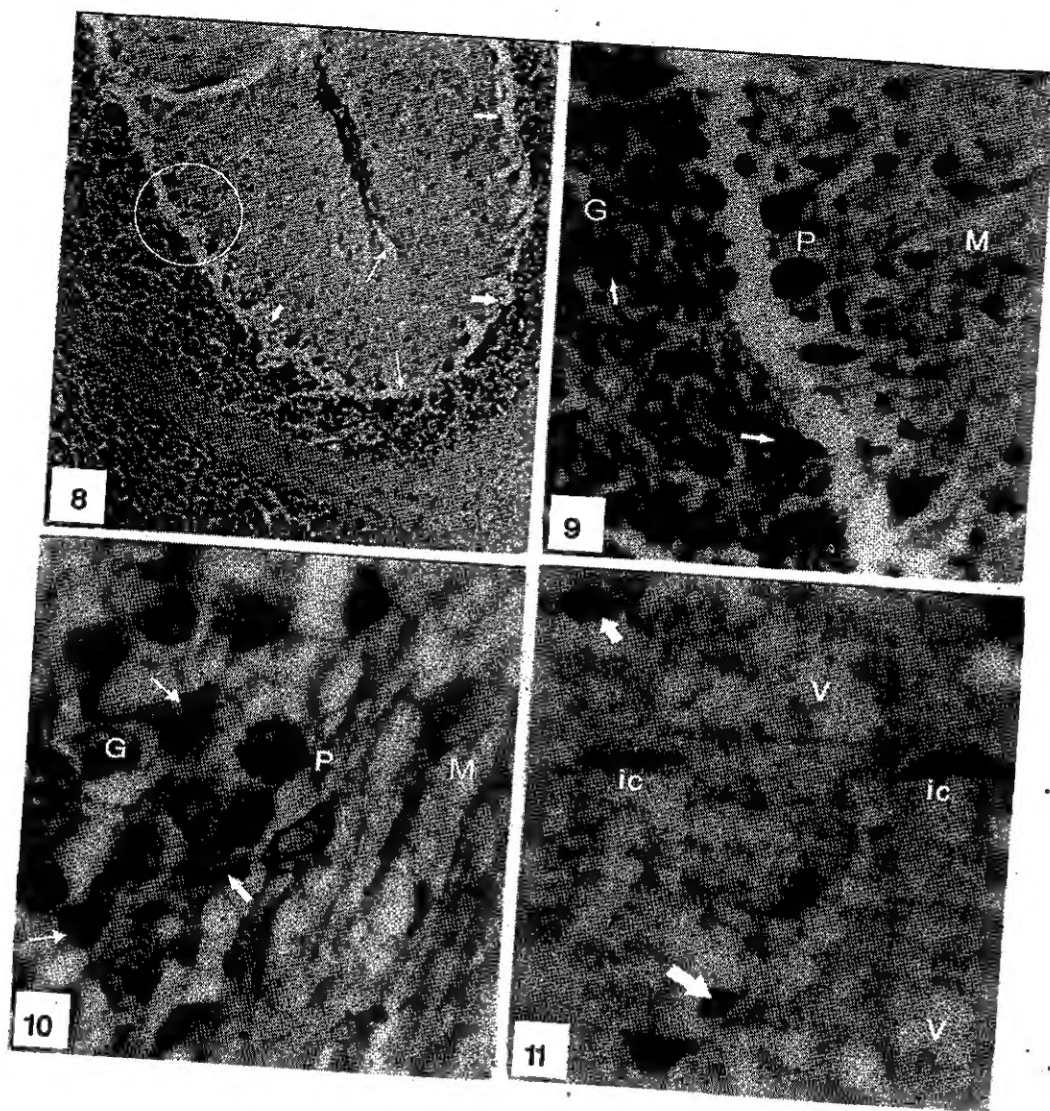
Fig. (3): Showing the characteristic pyriform shape of Purkinje cells (P) containing well-formed peripheral nuclei with prominent and conspicuous nucleoli and their cytoplasm is relatively and strongly stained ( $\times 1000$ ).

Plate-II



- Photomicrographs (Figs. 4-7) of sections in cerebellar cortex of irradiated ♀ rabbits stained with H. & E.:**
- Fig.(4): Showing herniation of the granular layer (G) loss of Purkinje cells (arrows) and detaching of pial surface (s) epithelia ( $\times 200$ ).
- Fig.(5): Showing 3 Purkinje cells ( $P_1$ - $P_3$ ) at different stages of degeneration, degenerated and accumulated granular cells (G) and vacuolated molecular (M) layer ( $\times 400$ ).
- Fig.(6): Showing edematous spaces (ES) congested with blood and highly degenerated (arrows) Purkinje cell area. Two destructed Purkinje (P) cells ( $\times 400$ ).
- Fig.(7): Showing a dark Purkinje cell (DP), edematous spaces (ES), glioses (GI) and widened pericellular spaces (PS) ( $\times 1000$ ).

Plate-III



**Photomicrographs (Figs. 8-11) of sections in cerebellar cortex of irradiated ♂ rabbits stained with H&E.:**

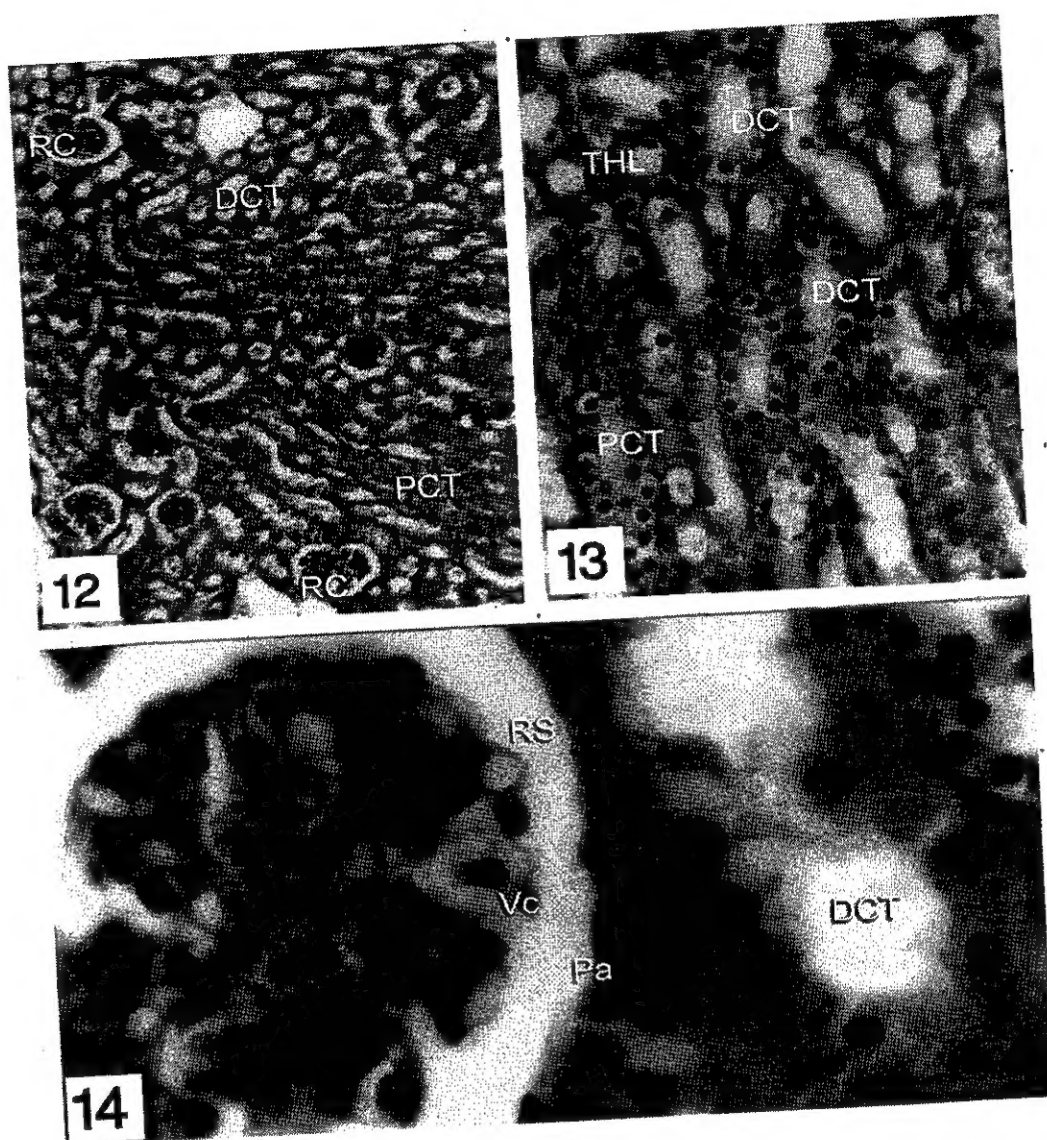
Fig.(8): Showing part of the cerebellar folium demonstrating some sort of cavitation (thin arrows) inside the cortical layers and between the molecular and granular layers ( $\times 200$ ) with degeneration and absence of Purkinje cells at several sites of their layer (thick arrows).

Fig.(9): A magnified part ( $\times 400$ ) of the previous field (encircled) showing clusters of degenerated granular cells (arrows), four dark Purkinje cells (P) and vacuolated molecular (M) layer ( $\times 400$ ).

Fig.(10): Another magnified part ( $\times 1000$ ) of Fig. (8): The cortical layers containing highly destroyed Purkinje cells (P) and granular cells (G), perineural and perigial edema (arrows).

Fig. (11): A magnified part ( $\times 1000$ ) of Fig. (8) showing highly vacuolated molecular layer (v) and pyknotic nuclei of damaged cells (arrows) and infiltrated cells (ic).

Plate IV



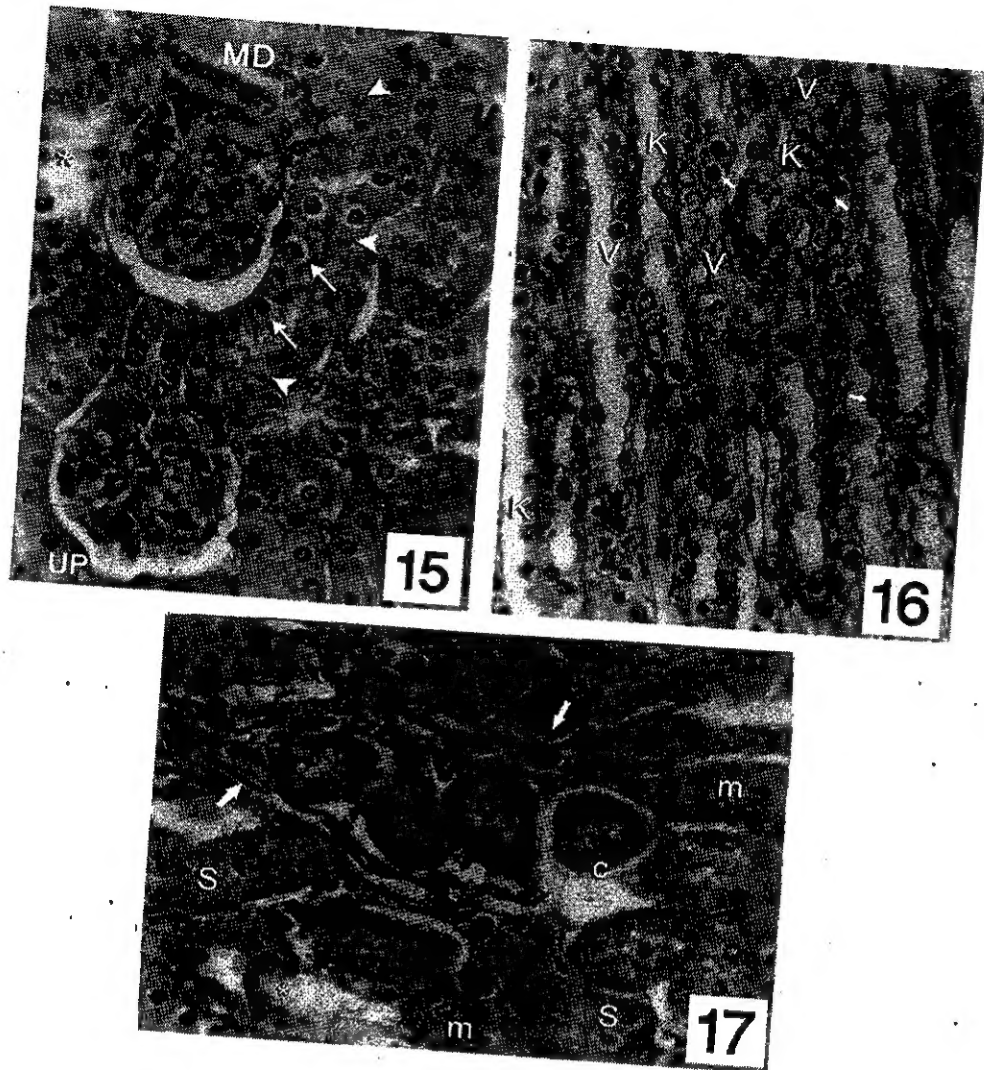
**Photomicrographs (Figs. 12-14) of sections in kidney of normal rabbit (H&E).**

Fig. (12): Showing the characteristic elements of the normal histology of kidney (X 100). Renal corpuscles (RC), Proximal Convoluted Tubules (PCT), Distal convoluted Tubules (DCT).

Fig. (13): Showing a part of the renal cortex and renal medulla demonstrating proximal (PCT), distal convoluted tubules (DCT) and the thin limbs of Henle's loops (THL) with prominent nuclei (X 400).

Fig. (14): Showing a magnified part (X 1000) of the renal cortex with a glomerulus composed of a number of capillary loops together with few mesangial cells, podocytes visceral (Vc) and parietal (Pa) layers of Bowman's capsule and normal renal space (RS). In the field a distal convoluted tubules (DCT) with large lumen and lining epithelia having prominent nuclei was demonstrated.

Plate V



- Photomicrographs (Figs. 15-17) of sections in kidney of irradiated ♀ rabbit (H&E ×400):**
- Fig. (15): Showing a part of the renal cortex revealing hypertrophied glomeruli with degenerated and pyknotic nuclei, DCT cells with degenerated cytoplasm and signs of karyolysis in some of their nuclei(\*), some PCT cells showed pyknotic nuclei (→), while others showed degenerated cytoplasm and nuclei (arrow heads). Notice, the adhesion between the glomerular tuft and Bowman's capsule and the degenerated nuclei of macula densa (MD). The urinary pole (UP) is also demonstrated.
- Fig. (16): showing Henle's loops of renal medulla exhibiting partial cellular necrosis with some cells contained pyknotic nuclei (arrows). Others showed signs of karyorrhexis (K) and vacuolated cytoplasm (V).
- Fig. (17): Showing another part of renal cortex with tubular collapse, moderate inflammatory infiltrate in the interstitium (arrows). DCT(\*) with pyknotic nuclei and destroyed cells, PCT cells showing marginal chromatin (m) or condensed chromatin (C) or karyolysed nuclei (S).

Plate VI

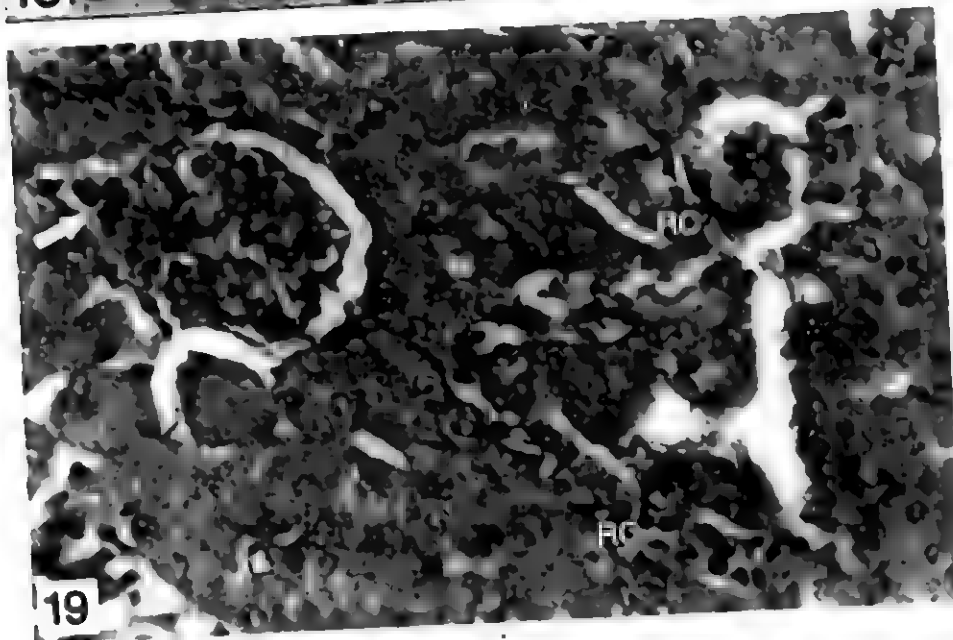
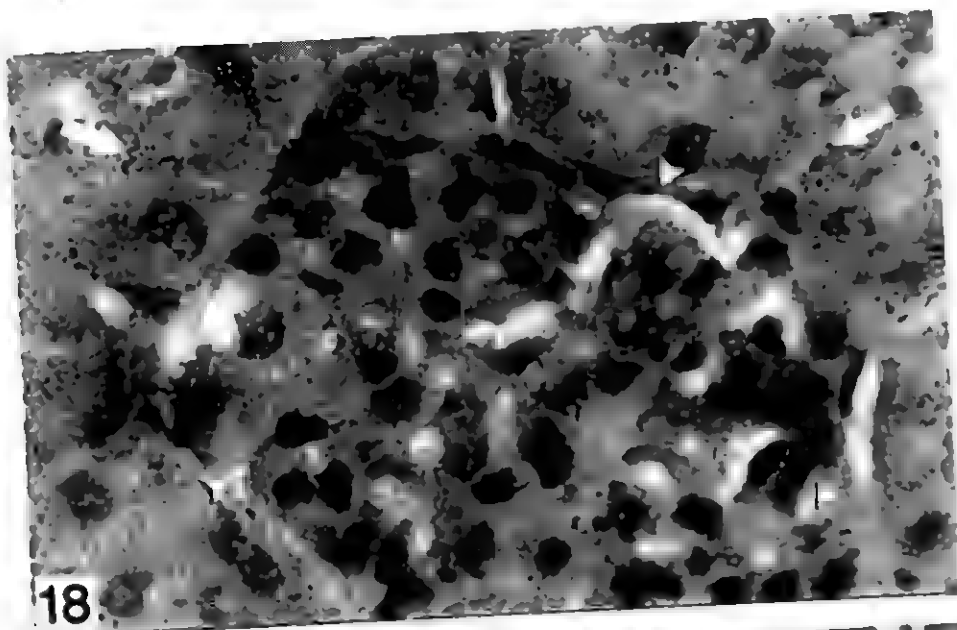
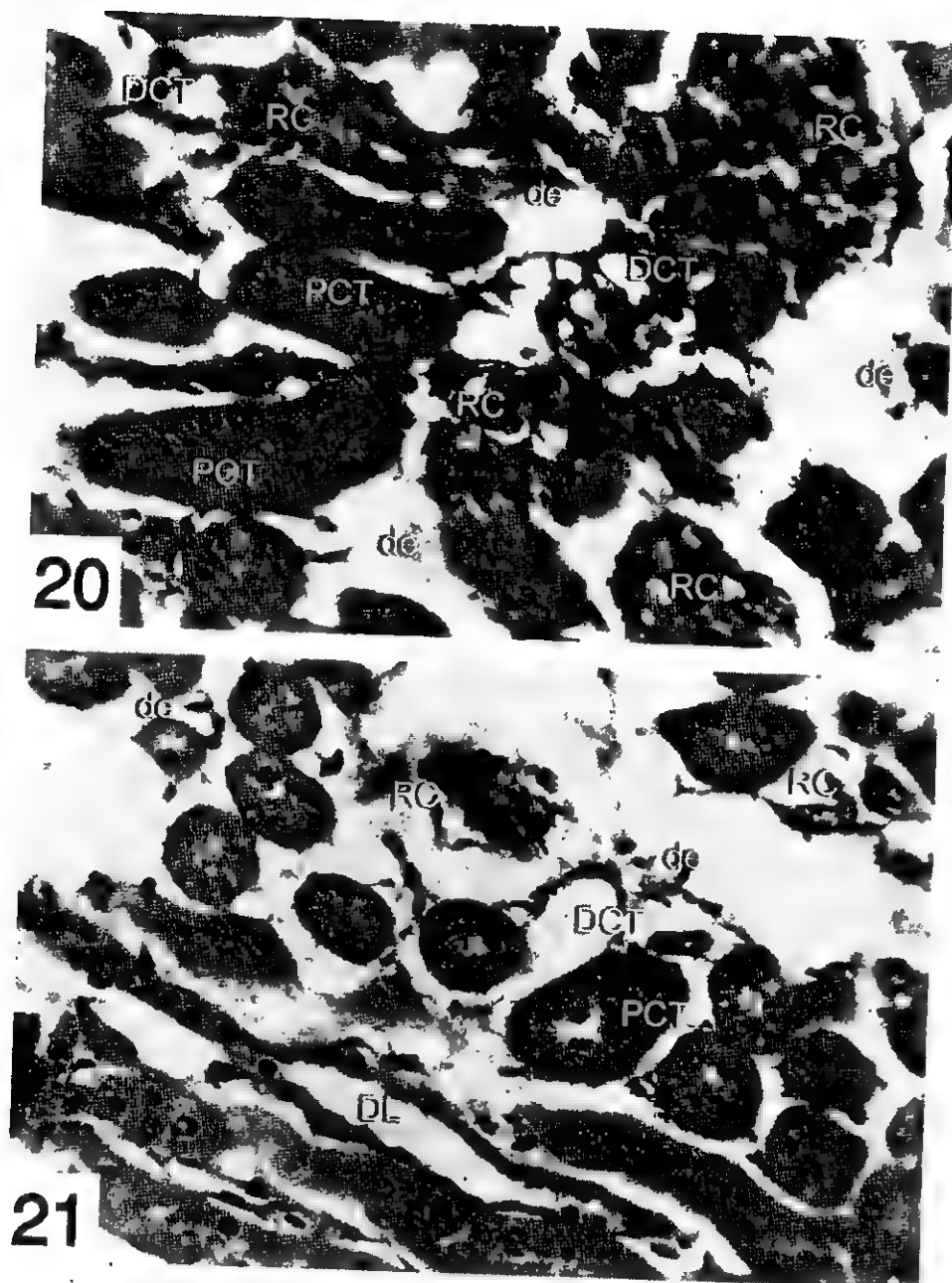


Fig. (18) A photomicrograph of a section in kidney of irradiated ♀ rabbit (H&E  $\times 1000$ ) showing vacuolar degeneration of a hypertrophied renal corpuscle with a highly affected visceral and parietal layers (arrow heads), degenerated and pyknotic nuclei and absence of renal space. The renal epithelia appears with necrotic remnants (arrows).

Fig. (19) A photomicrograph of a section in kidney of irradiated ♂ rabbits (H&E  $\times 400$ ) showing glomerular sclerosis (arrow), tubular necrosis and collapse and another two highly degenerated renal corpuscles (RC).

Plate VII



**Photomicrographs (Figs. 20-21) of sections in kidney of irradiated ♂ rabbits (H&E×400):**  
 Fig. (20) Showing severely damaged renal corpuscles (RC), pronounced tubular atrophy with highly widened intertubular spaces and absence of normal tubular epithelia. The DCT are more destructed than PCT. Notice the cellular debris (de).  
 Fig. (21) Showing another field of renal cortex demonstrated severely damaged renal corpuscles (Rc). Highly atrophied and necrotic DCT, PCT and descending limbs (DL) of Henle's loops widened intertubular spaces and cellular debris (de).

## Discussion

There is a recognized need for understanding the effects of microwaves on humans and animals due to the rapidly expanding exposure of the public to domestic and industrial applications of microwave technology. Therefore, in an attempt to depate or detect any biological effects of these non-ionizing radiations on the general health, it is an expected point to investigate their effects on some target organs such as cerebellum and kidney in an experimental mammalian animal. Since most of the mobile phone users carrying it attached to their belts, very close to the kidneys. Therefore, the emitted radiations of the mobile phone may be absorbed by the kidney more than the other internal organs. It was also previously concluded that, the kidney was a major potential route for the absorption of hazardous materials encountered in the environment (*Irmak et al., 2002*). On the other hand, we designed this experiment to investigate the effects of these non-ionizing radiations on the domestic rabbit as a mammalian animal in the premature stage. The reason of choice of this stage is that they are comparable to human mobile phone addicted teen-agers with respect to age (*Salford et al., 2003*). The intense use of mobile phones by youngsters is a serious memento since the developing organs are more vulnerable than mature organs to microwave radiations because of their lower capacity to dissipate the non-ionizing radiation (*Michaelson, 1982*). The present study suggested that, the exposure of the experimental animals to the microwaves of the mobile phone at a frequency of 900 MHz and SAR of 0.62 W/Kg resulted in different histopathological changes which could be recorded allover the three cortical layers of rabbit cerebellum. The degenerative effects of microwave irradiation were more destructive in exposed males than exposed females. Concerning the gross histological structure of cerebellum some sort of herniation and cavitation in some cerebellar folia could be recorded in this experiment. This herniation and cavitation may be attributed to excessive destruction of cells

and increased accumulation of damaged cells inside the granular layer where its cells have the capacity to engulf the other degenerated cells (*Albert and Sherif, 1988*). This comes in disagreement with the results of *Gona et al. (1993)* who reported that there was no herniation or cavitation and the pattern of foliation as well as the width and thickness of the four layers of cerebellar cortex were similar in two groups of newly born rats. The first group was control pups, while the other was irradiated pups exposed to 60 Hz electric and magnetic fields. In the present study the granular cells revealed several histopathological criteria after radiation exposure. The neurons of this layer appeared greatly damaged, clumped, aggregated and surrounded with edematous spaces. The latter resulted into compression of the neuronal mass leading to cellular crowding and gliosis. These changes may be attributed to changes in fluid osmolarity in cerebellum as evidenced by *Singh and Singh (2002)* or may be due to mechanical effects related to increased local blood flow as suggested by *Oscar et al. (1981)*. *Singh and Singh (2002)* concluded that, the changes in water structure, produced by magnetic fields may have inhibitory effects by changing the cytoplasmic organization, structural chemistry and activities of the extracellular and intracellular fluid components. This has been responsible for producing edema in the cerebellum due to changes in fluid osmolarity. This edema of the intracellular matrix has been in turn responsible for producing compressive degeneration of the affected cells resulting into apoptosis. While, *Oscar et al. (1981)* suggested that the effects of microwave exposure on the blood brain barrier (BBB) permeability are related to an increase in the local cerebral blood flow. The mechanical effect of hypertension and vasodilatation may also play a direct effect or indirect role. Also, a potential gliosis was recorded previously in the rat brain by *Ammari et al. (2008)* as a result of chronic exposure to GSM 900 MHz microwaves. In the current study, the observed generalized

spongiform changes which resulted in degenerative changes in neurons in the three cortical layers of cerebellum of exposed animals also extend and support similar results recorded by *Singh and Singh (2002)*. The authors recorded those results in adult male rats of Charles Foster strain after giving them magnetized water for drinking add libitum for a period of 30 days. In the present work, the histological observations recorded the presence of dark neurons in the granular and Purkinje cell layers of cerebellum of exposed animals of both sexes. These dark neurons appeared as small deeply-stained cells with hyperchromatic pyknotic nuclei within the granular layer. They also appeared as dark Purkinje cells which sometimes having nuclear and cytoplasmic degeneration with distinct chromophilia or having ill-defined shapes without any cellular details. Similar observations were noticed by several investigators (*Albert and Sherif, 1988; Fritsch et al., 1994; Majno and Joris, 1995; Salford et al., 2003; Hany, 2003 and Bertil et al., 2005*). *Albert and Sherif (1988)* studied the effect of the exposure of rats to microwaves for 90 consecutive days, twenty minutes per day on the cerebellar cortex. They recorded the presence of these dark neurons as small deeply- stained cells within the granular layer by the light microscope. They confirmed this observation by the electron microscope. According to *Fritsch et al. (1994)* these dark neurons might be a manifestation of the beginning of neuronal damage. According to *Majno and Joris (1995)* the dark staining of these damaged and degenerated neurons reflects the accumulation of denatured proteins and this might be caused by failure of the antioxidant system and uncompensated oxidative stress. While *Salford et al. (2003)*, found a highly significant evidence for neuronal damage caused by non-thermal microwave exposure. The cortex as well as the hippocampus and the basal ganglia in the brains of exposed rats contain dark neurons. Also, *Hany (2003)* noticed these dark neurons in the cerebellar cortex of rats exposed to microwaves of mobile phone. He stated that the observation of these dark neurons in the Purkinje and granular layers is suggestive of both types of cell

degeneration. According to *Bertil et al. (2005)* the appearance of these dark neurons in the brain of rats exposed to mobile phone microwaves (915 MHz) was clearly more frequent in exposed than unexposed animals. They were used this as an indicator of neuronal damage, both within the nucleus and the soma, in the long run resulting in neuronal shrinkage. It is possible to apply all these explanations to the observed changes in the present study. On the other hand, some investigators considered these dark neurons to be some sort of artifact in the fixation process (*Scherini et al., 1981*) or may be due to the different affinities of Purkinje and granular neurons for staining which depends upon the physiological status of the cells at the time of fixation (*Boselova et al., 1978*). However, in the present study the use of the same laboratory methodology for the normal control and exposed groups of animals can counteract these possibilities. Also in the present experiment, the histological observations revealed a detectable loss in the neurons of Purkinje and granular cell layer as a result of the destructive and degenerative changes of microwave exposure. This loss was readily apparent in Purkinje cell layer as an empty spaces or discontinuity in their linear arrangement, but in the granular layer appeared as pericellular and empty spaces. This was explained previously by *Brodal and Bajaalie (1997)* who considered that the cerebellar granular neurons are the most sensitive neurons for external stimuli. Another explanation was introduced by *Mark et al. (1997)* who attributed the loss of these cerebellar neurons to degenerative changes accumulating in neurons when being exposed to stressful situations. The observed empty spaces and increased and widened pericellular spaces surrounding the Purkinje and granular cells in the present study were previously recorded also by other authors (*Mark et al., 1997*). They reported that the presence of multiple empty spaces is a major sign of cell degeneration. The terminal step of the changes is the neuronal degeneration. It seems reasonable to apply this explanation to the recorded observations in the current study.

The close proximity of the antenna of the mobile phone to the abdominal

organs has raised concerns about the biological interactions between EMR and the kidney. The results of the present study indicated that rabbits exposed to 900MHz radiofrequency radiation develop renal lesions. Initially under the conditions of the present experiment both of glomeruli and tubules seemed to be sensitive to the radiation damage. In our study the different histological lesions which could be recorded in the kidney of rabbits of both sexes, as a result of exposure to mobile phone radiation, revealed that the renal tissue of tubules is more sensitive to the non-ionizing radiations than that of the glomeruli and stromal cells. These observations come in consistence with those of *Inaloz et al. (1997b)* who recorded similar results in pregnant and newborn rat's kidney after the exposure to microwave oven radiation. The vacuolar degeneration in the glomeruli and renal tubules of the kidney in the present experiment supports and extends similar observations recorded by *Nergiz et al. (2000)* who recorded extensive apical vesiculation in the renal tubular epithelia obliterating the lumen with degenerative changes in kidney glomeruli. While the state of renal tubular atrophy and collapse with contained necrotic remnants which could be recorded in the present results in the renal tissue of exposed female rabbits come on line with those of *Accinni et al. (1988)* who recorded collapsed tubules in the renal tissue of animals exposed to mobile phone microwaves. Also, the degenerative changes recorded in the renal tissue of the present experimental animals with glomerular sclerosis and renal tubular atrophy confirm the previous data of *Nergiz et al. (2000)* who recorded glomerular sclerosis as an end stage of degenerative changes and attributed this to the extensive increase in collagen fiber bundles. Also, *Accinni et al. (1988)* suggested that the glomerular changes produced in rabbit kidney due to radiofrequency radiation exposure (27.12-MHz) were induced by the localization of antigen-antibody complexes. The nephrities induced by ionizing radiation is a very well documented clinical entity that occurs in a proportion of individuals whose kidneys have been irradiated by a certain critical dose in the

course of x-ray therapy for a year by neoplasm (*Madrazo and Churg, 1976*). Several clinical and experimental studies have described pathological findings in kidney damaged by x-irradiation and the mode of their progression which does not seem to be sustained by immunological mechanisms (*Keane et al., 1976 and Williams, 1986*). Although these two types of radiation nephrities evolve differently, i.e., toward glomerulo-sclerosis and interstitial fibrosis in ionizing radiation nephrities and toward glomerular membranous changes associated with immune deposits in non-ionizing radiation nephrities. There are striking similarities of lesions recorded in the present study and those of the previously mentioned authors. Although the mechanisms by which ionizing and non-ionizing types of radiation induce these changes are not clear. The similarity of the lesions recorded in the present study with those of other investigators suggests that a common pathogenetic mechanism may be operative yet, a common reaction of the renal structures to different pathogenetic mechanisms can not be excluded. The histopathological lesions recorded in irradiated males were more prominent and more drastic than those recorded in the irradiated females. The subject which may be attributed to the radioprotective effect of the female sex hormones as recorded previously by other investigators (*Rugh, 1966*).

## References

1. **Accinni L, De Martino C and Mariutti G (1988):** Effects of radiation on rabbit kidney : morphological and immunological study. *Exp. Mol. Pathol.*, 49 (1): 22-37
2. **Albert E and Sherif M (1988):** Morphological changes in cerebellum of neonatal rats exposed to 2.45 GHz microwaves. *Prog. Clin Biol. Res*; 257:135-151.
3. **Ammari M, Brillaud E, Gamez C, Lecomte A, Mohsen S, Abdelmelek H and De Seze R (2008):** Effect of a chronic GSM 900 MHz exposure on glia in the rat brain. *Biomedicine & Pharmacotherapy*, 20: 1-9
4. **Athina P, Vossiliki K, Angeliki C, Prodromos H, Eleni N, Ionnis N, Thomas D X, Theodoros D, and Georgios K**

- (2004): Bone morphogenetic protein expression in newborn rat kidneys after prenatal exposure to radiofrequency radiation. *Bioelectromagnetics* 25:216-227.
5. Bancroft J D and Gamble M (2002): *Theory and Practice of Histological Techniques*, 5<sup>th</sup> Edition, Churchill, Livingstone, London.
6. Bertil R R S, Jacob E, Lars M, Mikael B P, Arne B and leif G S (2005): Effects of microwaves from GSM mobile phones on the blood brain barrier and neurons in rat brain. *Progress In Electromagnetics Research Symposium*, Hangzhou, Chian, August 22-26, pp.1-3.
7. Boselova L, Ochodnicka E, Magdolenva S, Moravci-Kova Y and Meitner E R (1978): The Golgi apparatus of pale and dark Purkinje cells. *Folia Morphol.*, 26:257-259.
8. Brodal P and Bajaalie, J G (1997): Salient anatomic features of the corticoponto-cerebellar pathway. *Prog. Brain Res.*, 14: 227-249.
9. Fritsch P, Richard-Le Naour H, Denis S I and Ménétrier F (1994): Kinetics of radiation-induced apoptosis in the cerebellum of 14-day-old rats after acute or during continuous exposure. *Int. J. Radiat. Biol.*, 66(1): 111-117.
10. Fritze K, Sommer C, Schmitz B, Mies G, Hossman K. A, Klessling M and Wiessner C (1997): Effect of global system for mobile communication (GSM) microwave exposure on blood-brain permeability in rat. *Acta. Neuropathol.*, 94 (5): 465-470.
11. Gona A G, Yu M C, Gona O, al-Rabiai S, Von Hagen S and Cohen E (1993): Effects of 60 Hz electric and magnetic fields on the development of the rat cerebellum. *Bioelectromagnetics*, 14 (5): 433-447.
12. Hany M H M (2003): Changes produced by the exposure to microwaves of mobile phone on cerebellum of albino rat. Ph. D. Thesis, Anatomy Department, Faculty of Medicine, Assiut University.
13. Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M, Akyold O and Ozen S (2004): Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain. *Clinica. Chimica. Acta.* 340 :153-162.
14. Inaloz S, Dasdag S, Ceviz A and Bilici A (1997a): Acceptable radiation leakage of microwave ovens on pregnant and newborn rat brains. *Clin. Exp. Obstet. Gynecol.*, 24(4):215-9.
15. Inaloz S, Dasdag S, Aslan A, Bilici A and Yayla M (1997b): The effects of the microwave oven on pregnant and newborn rat kidneys. *Urogynaecologia International Journal*, 11 (1): 9 - 15.
16. Irmak M, Fadillioglu E, Gulec M, Erdogan H, Yagmurca M and Akyol O (2002): Effects of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. *Cell biochem. Funct.* 20: 279-283.
17. Keane W F, Crosson J T, Staley N A, Anderson W R and Shapiro F L (1976): Radiation - induced renal disease . A Clinicopathologic study . *Am. J. Med.* 60:127-137.
18. La Regina M, Moros E G, Pickard W F, Straub W L, Baty J and Roti Roti J L (2003): The effect of chronic exposure to 835.62 MHz FDMA of 847.74 MHz CDMA radiofrequency radiation on the incidence of spontaneous tumors in rats. *Radiat. Res.*, 160(2): 143-151
19. Madrazo A A, and Churg J (1976): Radiation nephritis. Chronic changes following moderate doses of radiation. *Lab. Invest.*, 34:283-290.
20. Majno G and Joris I ( 1995) :Apoptosis, oncosis, and necrosis. An overview of cell death. *Am. J. Pathol.* ;146(1):3-15.
21. Mark R E, Griffin S T and Graham D I (1997) :Aging-associated changes in human brain. *J. Neuropathol. Exp. Neurol.*, 56:1269-1275.
22. Meral I, Mert H Mert N, Deger Y, Yoruk I and Yetkin A (2007): Effects of 900 MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels of guinea pigs. *Brain Res.*, 1169: 120-124.
23. Michaelson S M (1982) :Microwave and radiofrequency radiation in: M.J. Suess (ed.): *Nonionizing Radiation Protection*. WHO Regional Publications . European series (10) Copenhagen:97-174.
24. Nergiz Y, Ketani M, Akday Z, Ersay A and Celik M (2000): Effect of low-intensity microwave radiation on rat kidney: an ultrastructural study. *Turk. J. Med. Sci.*, 30 (3): 229-234.
25. Oscar K J, Gruenau S P, Folker M T and Rapoport S I (1981): Local cerebral blood flow after microwave exposure. *Brain Res.*, 204: 220-225.
26. Ozguner F, Oktem F, Ayara A, Koyu A and Yilmaz H R (2005): A novel antioxidant agent caffeic acid phenethyl ester prevents long-term mobile phone exposure-induced renal impairment in rat. *Molecular and Cellular Biochemistry*, 277: 73-80.
27. Rugh R (1966) : *Hand Buck Der Med. Radiol. Strahlenbiologie* , vol. 11. Berlin.

28. Salford L G, Brun A E, Eberhardt J L, Malmgren L and Persson B R (2003 ): Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. *Environ. Health Perspectives*, 111(7):881-883 .
29. Scherini E, Bolclii F, Biggiogera M and Bernoccfii G (1981): Further evidence of different morphofunctional aspects in the Purkinje cell population of adult rat cerebellum. An ultrastructural study. *J. Submicrosc. Cytol.*;13:17-29.
30. Sanchez S, Masuda H, Billaudel B, Haro E, Anane R and Leveque P (2006): Effect of GSM-900 and 1800 signals on the skin of hairless rats. 11: 12 week chronic exposures. *Int. J. Radiat. Biol.*; 82(9): 675-680.
31. Shirai T, Ichihara T, Wake K, Watanabe S, Yamanaka Y and Kawabe M (2007): Lack of promoting effects of chronic exposure to 1.95 GHz W. CDMA signals for IMT-2000 cellular system on development of N-ethylnitrosourea-induced central nervous system tumors in F344 rats *Bioelectromagnetics*, 28 (7): 562-572.
32. Singh M and Singh K P (2002): Adult rat brain changes induced by magnetised water. *J. Anat. Soc. India*, 51(1): 47-49.
33. Williams M V (1986) : The cellular basis of renal injury by radiation. *Brit. J. Cancer*, 53, VII : 257-264.

## التغيرات الناتجة في مخيخ وكلية الأرنب بعد التعرض المزمن لإشعاعات الهاتف النقال دراسة هستوباثولوجية

سمير عبد العظيم نصار\* ، مريم عمران شكورفو\*\*

\* قسم علم الحيوان، كلية العلوم، جامعة الزقازيق، مصر.

\*\* قسم علوم الحياة، كلية الآداب والعلوم (الخمس)، جامعة المرقب، ليبيا

يعتبر استعمال الهاتف النقال في الوقت الحالي من أسرع التقنيات الحديثة نمواً وتطوراً. وتعتبر الأشعة الصادرة عن هذا الجهاز نوعاً من أنواع الإشعاعات غير المؤينة التي ما زالت تحظى بالكثير من الشكوك حول خطورتها في حالات التعرض الحاد والمزمن.

ولم تلقى التأثيرات البيولوجية المباشرة نتيجة التعرض لهذا النوع من الإشعاعات غير المؤينة قدراً كافياً من الدراسة المكثفة خصوصاً من ناحية تأثيراتها الهستولوجية. نظراً للقرب الشديد لهذا الجهاز أثناء استعماله لمنطقة الرأس والأعضاء البطنية تصاعدت المخاوف والاحتمالات حول التفاعلات البيولوجية لهذه الإشعاعات الكهرومغناطيسية مع المخيخ والكلية فوقع عليهما الاختيار ليكونا بمثابة الأعضاء المستهدفة لدراسة المخاطر المحتملة للإشعاعات الصادرة من هذا الجهاز. وتهدف الدراسة الحالية لبحث وتقييم التأثيرات الهستوباثولوجية للتعرض المزمن المتكرر لهذه الإشعاعات على نسيج المخيخ والكلية لحيوان ثديي مثل الأرنب المنزلي. واستعملنا في هذه الدراسة ذكور وإناث الأرنب التي قسمت إلى ثلاث مجموعات على النحو التالي: المجموعة النمطية الضابطة ( $\sigma + \phi$ )، المجموعة المعرضة للإشعاع من الإناث ( $\phi$ )، المجموعة المعرضة للإشعاع من الذكور ( $\sigma$ ). وتم اختيار الحيوانات كلها في مرحلة ما قبل النضج الجنسي وعدم ظهور أي أعضاء جنسية خارجية عند بداية التعرض للإشعاع حيث يوازي هذا العمر في الحيوانات عمر الشباب من بني البشر في سن 11، 12، 13... المولعين باستخدام الهاتف النقال. كما أن الأعضاء الصغيرة غير الناضجة من المحتمل أن تكون أكثر تأثراً بهذه الإشعاعات من الأعضاء الناضجة وذلك لعدم قدرتها على مجابهة الإشعاعات غير المؤينة وتحمل آثارها الضارة. وتم تعريض الحيوانات لإشعاعات الهاتف النقال ذات التردد 900MHz لفترات كانت 30 دقيقة/اليوم ولدة 90 يوماً في حالة المخيخ، 90 دقيقة/اليوم ولدة 90 يوماً في حالة الكلية غير أن الهاتف المعلق بالقرب من الكلية يعمل في وضع استعداد فقط ولا يتلقى إشارة من الهاتف الآخر. بفحص المقاطع النسيجية المأخوذة من حيوانات التجارب المعرضة للإشعاع والمصبوغة بصبغ الهيماتوكسيلين والإيوسين بالمجهر الضوئي أظهرت النتائج ما يلي: في نسيج المخيخ تم تسجيل علامات واضحة للفتق الدماغي وانفصال للطلائية المكونة للسطح الحنون مع مظهر عام من التورم والاحتقان حول الأوعية الدموية والخلايا العصبية وخلايا الغراء العصبي. وفي طبقة بركنج أظهر هذا النوع من الخلايا العلامات المرضية الآتية: تهمد وعدم انتظام الخلايا في طبقة خطية ونقص شديد في تواجدهم وأحياناً ظهرت الخلايا محاطة بخلايا محببة شديدة التلف وفراغات محتقنة بالدم، وفي أحيان أخرى ظهرت خلايا بيركنج داكنة وغير منتظمة الشكل وصغيرة في الحجم عن الوضع الطبيعي وفقيرة التمييز. وكثيراً ما ظهرت محاطة بفراغات حول خلوية متسعة نتجت من تكسير وتلف الخلايا

المحيطة، وينتهي بها المطاف بالغياب والاختفاء التام من بعض المناطق في نسيج المخيخ بعد زيادة حالة التلف والتهدم. وفي خلايا الطبقة المحيطة ظهرت هذه الخلايا شديدة الاصطباج في مجموعات تحت طبقة خلايا بيركنج وأحياناً أخرى تتهدم وتلتصق بخلايا الغراء العصبي فتظهر على شكل كتل متهدمة من خلايا الغراء العصبي، وفي مقاطع نسيجية أخرى ظهرت صغيرة الحجم مع بكنزة واضحة وزيادة الاصطباج في أنويتها وفي مقاطع أخرى ظهرت بشكل إسفنجي عام متردي نتيجة التعرض الطويل للإشعاع. أما خلايا الطبقة الجزيئية وأليافها فظهرت أيضاً في حالة سيئة حيث بدت خلاياها متكسرة وظهرت المادة الخلالية لها ممثلة بالفراغات الهوائية بين الزوائد الشجرية للخلايا العصبية. وفي مقاطع أخرى ظهرت الطبقة الجزيئية يغزوها عدد من الخلايا المتكسرة وفي شكل إسفنجي عام يعكس حالة التلف والتردي لمكونات هذه الطبقة. في نسيج الكلى أظهر تعرض حيوانات التجارب لإشعاعات الهاتف النقال العديد من العلامات الباثولوجية التي تدل على خطورة هذه الإشعاعات منها: انتشار حالة التكسير والتلف في أنابيب الكلية القريبة والبعيدة، تهدم الكبيبات البولية، ضيق فراغ محفظة بومان، إسلتصاق واضح بين الخصلة الدموية للكبيبة البولية ومحفظة بومان، تلف جزئي في الأنابيب البولية، غزو خلايا الالتهاب للنسيج البيني، تقلص الأنابيب البولية واختفاء الفراغات البولية بداخلها حتى بدت وكأنها هياكل متقلصة مصمتة وليست كأنابيب، تفسخ للطلائية في الأنابيب البولية وأحياناً غياب كامل لها. وكانت الأنابيب الملتفة البعيدة أكثر حساسية وتأثراً بالإشعاع من الأنابيب الملتفة القريبة. وكانت هذه التأثيرات الهستوباثولوجية في كل من نسيج المخيخ والكلى لحيوانات التجارب المعرضة لأشعة الهاتف النقال أكثر حدة وظهوراً في الذكور عن الإناث.

تجميعاً لكل هذه النتائج يمكننا القول أن التعرض الطويل المزمّن لأشعة الهاتف النقال في حيوانات التجارب أدى الى ظهور تغيرات هستوباثولوجية واضحة في كل من نسيج المخيخ، الكلى، ويبدل ذلك على أن استعمال هذا الجهاز لفترات طويلة قد يشكل خطورة واضحة قد تصل إلى حد تسمم خلايا النسيج العصبي للمخيخ والنسيج البولي للكلى على الأقل عند ظروف تجربتنا الحالية وهي التعرض لمدة 30 دقيقة في اليوم لفترة 90 يوم في حالة المخيخ، مدة 90 دقيقة في اليوم لفترة 90 يوم في حالة الكلى.